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De- and Resensitisation of Cardiac β-Adrenergic Receptor Signaling:

A Modelling Approach

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Sammanfattning
Abstract:

Desensitisation is defined as a failure of a signaling pathway to respond to chronic or repeated stimulation. The β-adrenergic receptor signaling pathway of the healthy adult heart is known to desensitise, and then regain the sensitivity to stimulation if given enough time to rest between stimulations (resensitisation). The fetal heart does not desensitise, and in animal models of heart failure, a permanent desensitisation have been observed. No isolated element of the signaling pathway have yet been proven to be the sole modulator of the desensitisation behavior. Therefore a mathematical model of the signaling pathway has been constructed, minimized against theoretical desensitisation data and tested for resensitisation. The minimal models and the original model were capable of describing the theoretical de- and resensitisation of the pathway, and only one receptor type with three states was required in the minimal models, but one feedback from the kinases either to phosphorylation of the receptor or to breakdown of cAMP. The original model was also capable of describing experimental data of contraction force from chicken cardiac tissue. The cardiac tissue displays the peak behavior of the desensitisation when stimulated with ISO for ten minutes, and resensitises in less than 5 minutes.

Nyckelord
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1 Abstract

Desensitisation is defined as a failure of a signaling pathway to respond to chronic or repeated stimulation. The β-adrenergic receptor signaling pathway of the healthy adult heart is known to desensitise, and then regain the sensitivity to stimulation if given enough time to rest between stimulations (resensitisation). The fetal heart does not desensitise, and in animal models of heart failure, a permanent desensitisation have been observed. No isolated element of the signaling pathway have yet been proven to be the sole modulator of the desensitisation behavior. Therefore a mathematical model of the signaling pathway has been constructed, minimized against theoretical desensitisation data and tested for resensitisation. The minimal models and the original model were capable of describing the theoretical de- and resensitisation of the pathway, and only one receptor type with three states was required in the minimal models, but one feedback from the kinases either to phosphorylation of the receptor or to breakdown of cAMP. The original model was also capable of describing experimental data of contraction force from chicken cardiac tissue. The cardiac tissue displays the peak behavior of the desensitisation when stimulated with ISO for ten minutes, and resensitises in less than 5 minutes.

2 List of abbreviations

AC	Adenylyl cyclase
ATP	Adenosine triphoshpate
cAMP	Cyclic AMP or adenosine 3', 5' monophosphate
GPCR	G-protein coupled receptor
G-protein	Guanine nucleotide-binding protein
Gi	G-protein which inhibits AC activation
Gs	G-protein which stimulates AC activation
GRK	G-protein coupled receptor kinase
HEK 293	Human Embryonic Kidney 293 cells
ISO	Isoproterenol
ODE	Ordinary differential equation
PKA	Protein Kinase A

3 Introduction

This thesis introduces a mathematical model able to describe the de- and resensitisation of the cardiac β-adrenergic signaling pathway. The model is meant to be used in further studies of the difference between adult and fetal hearts in this pathway, as it has been shown that the desensitisation is prominent in adults, but lacking in fetuses and embryos [1].

Basic facts about the β-adrenergic signaling pathway and how it desensitises will be presented, followed by a general presentation of the philosophy and strategy of computational systems biology as well as a short summary of the existing models of the relevant pathways. Later sections of the thesis will treat the general strategies of modeling in more detail, present one specific model constructed by the author and describe how data to optimize this model was acquired. Last follows a discussion on the structure and predictions of the model, its limitations and its physiological implications.

3.1 Beta receptors at large A common mechanism for maintaining homeostasis in the body is through regulatory hormones and neurotransmitters. These substances are released from their site of manufacturing and travel to their target cell where they elicit a response. Once they have reached their target cell, they might pass the cell membrane, if their chemical structure allows it, and directly effect their target mechanism inside the cell. However, the most common way for them to work is to bind to the extracellular part of a receptor on the cell surface. The receptor in turn will then carry the signal into the cell through a signaling pathway that involves several different proteins.

The β-adrenergic receptors, or β-adrenoceptors, belong to the class of G-protein coupled receptors (GPCRs). This is one of the most numerous and well investigated classes of receptors, as well as the target of a large number of drugs. GPCRs consist of seven membrane spanning α-helices connected by intra- and extracellular loops, the loops determine the type of receptor. β-adrenergic receptors exist in three different types, of which two are common in the heart: β_1 and β_2, with β_1 being by far most abundant [33]. Examples of known agonists for the β_1 and β_2-adrenergic receptors are isoprenaline (ISO) and the catecholamines adrenaline and noradrenaline. When an agonist that can not cross the plasma membrane binds to the extracellular part of the receptor a conformational change occurs and a heterotrimeric guanine- nucleotide-binding regulatory protein (G-protein), bound to GTP and attached to the intracellular side of the receptor, is activated. The G-protein then disassociates into G_α and $G_\beta\gamma$ subunits. The sub-

units in turn will carry the signal further through different pathways. In this thesis we will consider the $G_{\alpha s}$ and $G_{\alpha i}$ pathways, which ultimately result in a change in force and speed of the contraction of the heart. In these pathways the G_α subunit affects adenylyl cyclase (AC). Depending on the type of G-protein, the α subunit will affect AC differently. $G_{\alpha s}$ will activate its catalytic properties, while $G_{\alpha i}$ will inhibit them. The signal from the G-protein is turned off when GTP is hydrolyzed to GDP, which then has to be replaced by a new GTP in order for the protein to be able to signal again. When AC is activated by $G_{\alpha s}$ it catalyzes the conversion of ATP to cAMP. cAMP is a common second messenger that serves many functions in the cell and a basal production is always present. It carries and modulates the signal through attaching a phosphate group to other proteins, a process called phosphorylation, inducing them to carry out their function. In order to remove the phosphate group, dephosphorylate the protein, another group of enzymes called phosphatases is required. The proteins phosphorylated by cAMP relevant for this model are the kinases protein kinase A (PKA) and G-protein coupled receptor kinases (GRKs). GRKs specifically phosphorylates the β-receptors, thereby preventing them from signaling through $G_{\alpha s}$. However, the β_2 receptor in it's phosphorylated state is able to signal through $G_{\alpha i}$ instead [2]. PKA is a more promiscuous protein than the GRKs and may phosphorylate the receptor, but also trigger breakdown of cAMP or carry the signal further down the pathway to elicit a cellular response, such as the opening of Ca^{2+} channels which will make the cell contract [3], or phosphorylate other receptors and inhibit pathways otherwise not connected to β adrenergic signaling (reviewed in [4] and [5]).

Once the receptor has been phosphorylated it may take one of several possible routes. It can be dephosphorylated and coupled to a new G-protein, ready to be activated again. It can also bind to β-arrestin, a protein which prevents reactivation of the receptor and additionally causes its internalization. Once internalized, the receptor can be dephosphorylated and recycled to the cell surface, or it can be fused to an endosome and degraded.

3.2 The desensitisation specifically Desensitization of β-adrenergic receptors in the adult heart is a complex process that happens in multiple steps, any of which may be referred to as simply "desensitisation" in the literature.

When the β-adrenergic receptor is first stimulated by an agonist, the cAMP concentration or the force of the muscle contraction (Figure 6) displays a peak, where the levels first rises considerably and then rapidly falls again to a new steady state (Isa Lindgen unpublished data, [7], [1]). This peak effect is typical of a negative feedback loop. Biologically it is thought to be mediated by phosphorylation

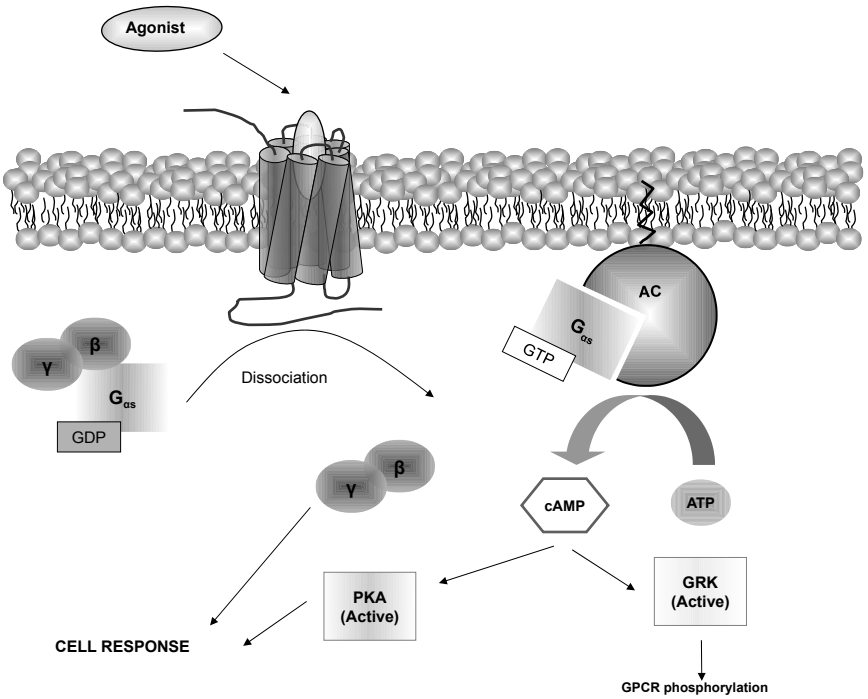


Figure 1: *Signaling pathway of the β_1-adrenergic receptor. Adapted with permission from [6].*

of the receptor through the kinases activated by cAMP. The duration of the desensitisation can be measured by a second activation only shortly after the first activation, when the response from the system will be much subdued [1], [7].

β-arrestin that binds to GRK phosphorylated receptors will facilitate internalization and downregulation of the receptor, which is the cause of the long-term desensitization seen in patients suffering from certain cardio- vascular conditions and in animal models of cardiac failure [8]. In congestive heart failure the heart is unable to eject blood as efficiently as required, and thus the tissues in the rest of the body will not get enough oxygen which will trigger release of cathecolamines. In such circumstances the levels of circulating catecholamines in the blood are high, but it does not help as a long-lasting desensitisation via a reduction of the density of β-adrenoceptors have been shown in both a rat model [9], in humans [10] [11] and in dog [12]. An elevation of G_i expression and functionhave been noted [13] [14], along with a proportional reduction of the expression and function of G_s and a decrease in AC expression and catalytic ability [15], which will

impair the signaling pathway. The desensitisation of the β adrenergic pathways precludes long-lasting treatment with β agonists in cardio- vascular conditions such as congestive heart failure.

Fetal hearts, however, do not desensitize at all but instead become sensitised when stimulated [1], with an increase in the expression and function of G_s and AC as well as a decrease in G_i expression and function[16] and changes in the β_1/β_2 ratio [4] [6] as compared to adult hearts. The fetal heart appears to be resistant to downregulation of the receptor in face of repeated stimulation[16]. Activation of fetal β-adrenoceptors also seem to desensitise other receptors who have an opposing effect on the cell [17].

The levels of circulating catecholamines during birth or hatching are very high, a necessary preparation for neonatal life that affects cardiovascular, respiratory, neurological and metabolic development. Slotkin *et al* [16] therefore hypothesize that the desensitisation, which would otherwise protect the heart against mechanical stress, has been programmed to develop postnatally in order to preserve these delicate developmental processes. However, it result could also be interpreted as the adult cells having lost some of the plasticity that is inherent of the developing cells.

What are the mechanisms behind the fetal lack of desensitization? This question is interesting enough on its own from a developmental perspective, but could knowledge gained from it also be used to help people with cardiac dysfunction?

3.3 Systems biology Carl Sagan said "The beauty of a living thing is not the atoms that go into it, but the way those atoms are put together." [18]. One of the most fascinating aspects of biology is the fact that components, many of which can not function on their own, when combined form something as complex and multifunctional as a living being. This prompts the thought that a component that shows one behavior or property on its own may show additional or completely new behaviors when part of a system. This is the basic thought for the field of systems biology, where experiences from engineering have been applied to biological systems in order to gain a deeper insight on how living things work.

Biological systems are often complex and large, with many parameters and intrinsically noisy. They frequently signal on multiple levels and perform multiple functions with several built-in control mechanisms. In computational systems biology, specialized computer software is used to build and simulate models of biological systems. With their superior computational power, these programs can help us gain an overview of systems that are otherwise hard to intuitively predict, offering interpretations and explanations that were not previously available. Mod-

els may also separate between different hypotheses or formulate new ones, which can be tested in the lab and the results used to improve the model, which will then formulate new hypotheses, and so on in an iterative process. *In silico* experiments are cheaper and simpler to run than wet lab ones, but the latter are still important to prove hypotheses. In order to make computer generated models useful they must be adapted to reality, and this is done by comparing their simulations to data from *in vivo* or *in vitro* experiments. Without this comparison, or optimization process, the model becomes nothing more than an extensive thought experiment.

There are many ways to build a mathematical model, and one must keep in mind that a model is always a simplification of the biological system studied. Therefore the strategy chosen for each model should reflect the question the model aims to answer. Ideally, the construction of a mathematical model in a systems biology setting is an iterative process where previous knowledge of the system is used to formulate the model structure, which is then fitted to experimental data and used to make predictions about the system. These predictions in turn can be used for the construction of new experiments whose data may be integrated in the model to make new predictions, which leads to new experiments, and so on. How the model is constructed is highly dependent upon the question to which we wish to apply it; a model can be quantitative or qualitative, constructed according to several different mathematical methods and very general to very detailed and specific, sometimes even able to zoom in on an area of interest.

The more detailed a model is, the more cumbersome, unstable and computationally heavy it becomes. In engineering one therefore generally seek to reduce the number of parameters as much as possible, while still keeping the desired behavior of the output intact. Such an *instrumentalistic model* can be achieved by equations which lack a biological equivalent, creating so called ”black boxes” for which we know the input and the output but not what is actually happening inside. Many biological systems are studied in a similar fashion: a stimulation is added and we measure the reaction of the organism but we can not truly see what is happening between the two events. This serves as reminder that several different systems structures may give the same difference in in- and out signal, and even though our model can be fitted perfectly to our experimental data, this is not proof that we have accurately described the system.

The instrumental approach is effective for constructing models that are useful for making predictions, but goes against the explanatory philosophy of biology. Biological models are often used to explore parts of and interactions in a system that are not directly measurable experimentally and this requires that many parameters are present that could otherwise have been omitted. As previously stated, this

type of *directly realistic models* carry certain problems, such as instability and a tendency to be over-parametrized and overfitted. For such an over parametrized system, there will be many parameter sets that carry acceptable solutions which might make it hard or even impossible to gain a clear view of the core predictions (for more on core predictions, see section 4.3).

A hybrid between these two approaches would yield a model which is *critically realistic*, where some output not measured in the lab have been chosen to be modeled and the model simulations are used to formulate explanations for their mechanistic behaviors [19]. The first model in this thesis is such a model. The signaling chain of the β-adrenoceptors have been simplified, with several different proteins lumped into the same state variable at times, but the basic structure of the signaling chain inside the cell has been preserved. A minimized model have then been used to identify crucial states and mechanisms for the desensitisation.

Analyzing a system can be done qualitatively or quantitatively. A qualitative analysis investigates whether a certain system's structure can describe the general attributes we are looking for, without specific units for measuring it. A quantitative analysis is more detailed, and evaluates whether it is possible to find a parameter set that can quantitatively describe the data, which would make it possible to predict specific values for the parameters, for example reaction rates.

3.4 Existing models of the β-adrenergic signaling pathway A large body of knowledge of the mechanisms of the different proteins in the β-adrenergic signaling pathway already exists, but in order for us to fully comprehend the functioning of the system, all these parts must be put together.

Several models which simulate different parts of the β-adrenoceptor signaling system already exist, a review of some of them can be found in [20]. Ligand binding of the receptor have been modeled, both in the aspect of how the ligand may bind to the receptor, receptor cross-linking, spatial distribution of the receptors and which effect different ligands have on the signaling pathway [21] [22] [23] [24] [25]. More and more focus has been put on compartmentation and spatial distribution of the signaling proteins, and here models can be of especially great use as practical experiments removing the compartmentation in the cell are difficult to perform [7], [26], [27], [28].

An ambitious model of the events from receptor activation all the way to cell contraction have been constructed by Saucerman et al [29], who later used a model to investigate how compartmentation affects signaling in neonate cardiomyocytes [30].

Several of these models are used in order to find more effective therapeutical

drugs in various forms of heart diseases. The model built in this thesis is aimed towards investigating the difference between the fetal and the adult β-adrenergic signaling.

3.5 Aim of the Thesis The model constructed in this thesis is meant to work as an overview of the β adrenergic signaling system in the adult heart, down to the level of cAMP and the kinase feedback loops. It is to be used as a basis for construction of experiments to further investigate the desensitisation of the same signaling pathway in adults and the possible reasons for the absence of the desensitisation in fetuses.

What we require from the model at this stage is that:

- It must be able to perform the peak effect behavior of the first stimulation.
- A second stimulation should show how long the duration of the desensitisation is.
- The desensitisation of the system should decrease more and more the longer the system is allowed to rest and finally disappear completely.
- There must exist at least one parameter set that can acceptably describe experimental data of the desensitisation gained from heart tissue.

4 Materials and methods

Here will follow descriptions of how the theories of mathematical modeling were applied to the β adrenergic receptor signaling pathway, as well as how data of its desensitisation was obtained from chicken hearts.

4.1 Computer software The software used in this thesis was Matlab R2009b with the add-on toolboxes SBTOOLBOX2 and SBPD [31]. Matlab is a technical computing language and environment designed by MathWorks to be used in solving a broad range of technical computing problems. Matlab in itself is very general-purpose, but several add-on toolboxes and packages can be downloaded to solve specific tasks or provide specific computing environments. SBTOOLBOX2 and its extension SBPD are Matlab add-on toolboxes specifically designed for constructing and simulating models in systems biology. The model was written and simulated in SBTOOLBOX2 and SBPD, and the optimization was constructed in the basic Matlab environment.

Code for the model and cost function are available in appendix.

4.2 Transforming biology to ordinary differential equations The *state* of a model can be described as a snapshot of it that contains all the informations needed to predict its actions. This information is contained in *state variables*, descriptions of how the different forms of the proteins react to signals within the system. The state variables of this model are each described by an ordinary differential equation (ODE), which tells us how the state variable changes over time, and an initial value, which gives us a starting point from which to begin counting.

An ODE can be defined as

$$\frac{d[x_i]}{dt} = f_i(x_1...x_n, p_1...p_n) \tag{1}$$

where x_i are state variables, p_i are parameters and t is time.

The ODEs in this model were built using the law of mass action, which states that the rate of an elementary reaction is proportional to the product of the concentrations of the participating substrates [32]. In order to calculate the change in concentration of a substrate (S) which turns into a product (P), with the help of a catalyst (C), one would multiply the concentrations of the substrate and the catalyst with a coefficient (k) which regulates the rate of the reaction. The rate can be dependent on many things, such as the proportions of the different proteins involved and the way in which they interact, and the constant may have different levels of complexity depending on the type of reaction. The equation for the simplest type of reaction involving the molecular species described above would look like this:

$$\frac{d[P]}{dt} = k_f * [S] * [C] \tag{2}$$

If it so happens that the product could also turn back into the substrate, this reaction must be subtracted from the previous equation to accurately describe the change of the system:

$$\frac{d[P]}{dt} = k_f * [S] * [C] - k_b * [P] \tag{3}$$

And the substrate would change correspondingly:

$$\frac{d[S]}{dt} = k_b * [P] - k_f * [S] * [C] \tag{4}$$

The concentrations of the different molecular species are then our state variables and the coefficients our parameters. The initial values will provide the proportions between different states of the same molecule, but not between different

molecules as the parameters are allowed to run free during the optimization (see 4.3).

An example from our model is the activation of the $G_{\alpha i}$ by the phosphorylated $\beta_2$receptor (see tab. 1)

$$\frac{d(Giact)}{dt} = k_8 * B2p * Gi - k_9 * Giact \tag{5}$$

A *variable* can be used to describe a relation between different state variables that is not affected by time, such as the number of receptors remaining the same, independently of which state they are currently in (see tab. 1).

$$B1tot = B1int + B1act + B1 + B1p \tag{6}$$

A complete list of the state variables in this model and their biological equivalent can be seen in tab. 1 and how they affect each other is depicted in Figure 2.

The values of the state variables are proportional and represent the proportion of the protein present in each state, not the absolute concentrations or amounts. Before the model was used to simulate any experiments, it was allowed to reach a *steady state* without an in signal. A steady state can be described as the typical state for the system to be in, where the concentrations of the different molecular species are in a dynamic equilibrium. Therefore the initial values play little role in the simulation of the experiments.

4.3 Optimizing the model When simulating the model, each state variable and each parameter needs to have a numerical value assigned to them. To help the model give as accurate a description as possible of our system, we need to fit it to our data. This is done through changing the parameters. Most often, values such as rate constants and concentrations in a biological system are described in the literature, but as it is rare for all constants in a system to be measured by the same research group in the same type of cell in the same experimental conditions, parameters gathered from the literature are likely to be inconsistent. This introduces an uncertainty in the model, and might compromise the simulation or give an inaccurate image of the system mechanics. Therefore we wish to employ another strategy to fit the model to the data; this is normally done through *optimizing* the parameters of our model. The optimization consists of constructing an optimization algorithm which strives to reduce the difference between the data points and the model simulation. A common way to do this is through a *cost function*. The cost function, as the name implies, calculates the *cost*, which is a measurement

of how much the model simulation deviates from the experimental data, often obtained through measuring the residuals. The larger the deviation, the larger the cost. The optimization algorithm will then strive to find combinations of values for the different parameters, *parameter sets*, which minimizes the cost as much as possible.

The cost function used in this thesis is

$$V(p) = \sum_{t=1}^{n} \frac{(y(t) - \hat{y}(t, p))^2}{SE(t)^2} \tag{7}$$

Where $V(p)$ is the cost for the model parameter set p, t is the time, $y(t)$ is the experimental data point at the time t and $\hat{y}(t, p)$ is the simulated value at the time t for the parameter set p. Acceptable parameter sets can then be determined through Pearson's χ^2 test as those that have a cost which is lower than χ^2(k), with k denoting the degrees of freedom. The cost is normalized with the standard error (SE) for each data point, which means that it will fit better to points with a small SE as they are considered to be more reliable. However, it is possible to introduce *weights* to the cost function which will force it to put more emphasis on points of our choice instead.

The optimization algorithm used was simannealingSBAOClustering from the Systems Biology Toolbox for Matlab. All parameter sets with a cost below the cut-off were saved and used in later analysis. Biological models are often over parameterized, which has the consequence that there might be more than one parameter set what has an acceptable fit to the experimental data. We would of course wish to use the best one, but how to define "best" when the mathematically optimal parameter set might make no sense biologically? As mentioned before, we could compare the optimized parameters to the literature to see if they are plausible, or even restrict which values they are allowed to take during the optimization. For these literature values to be relevant, they must be gathered from the same system under the same circumstances. Also, since all amounts of proteins used in this model are relative, the rate constants gained through the optimization will not be comparable to literature values.

Should no acceptable parameter set be found during the optimization we might have to reject the model structure altogether. This is one of the strongest statements that can be done in systems biology, and fits well together with the scientific notion that we can not prove hypotheses, only reject or fail to reject them [19].

4.4 Core predictions Core predictions are unique qualitative behaviors displayed by all acceptable parameter sets, that can later be measured experimentally.

Core predictions are acquired by simulating a new experiment with all or an intelligently chosen subset of the acceptable parameter sets, and if all parameter sets show the same behavior, we know that we have identified a mechanism essential for the function of the system [19]. The same procedure can be applied to state variables other than those optimized against to investigate possible essential mechanisms, but it is important that these predictions can be used to construct experiments to confirm or reject the hypothesis in order to be useful. When working with core predictions, we are not interested in the ”true” values of the parameters, as much as in the mechanisms of the entire system. Core predictions can be used to explore such questions as ”Is phosphorylation of the receptors necessary for the desensitisation?”, ”Are the elements already present in the signaling pathway enough to desensitise the system?” and ”Are both types of receptors essential for the desensitisation to occur?” rather than predict exact rate constants or concentrations.

4.5 Minimization of the model In order to see which states modulates the signal most significantly a minimized version of the model was constructed. This was done simply by excluding parts of the original model and optimizing it anew against the theoretical curve. If the reduced model was still able to describe the data, yet another state or a group of states were excluded. The final version will then be the simplest combination of states which can still describe the desensitisation.

4.6 Experimental procedures: contraction force of heart tissue To investigate the time span for the desensitisation in cardiac tissue, an *in vitro* experiment was performed. Due to restrictions in time and laboratory equipment cAMP could not be directly measured, but contractile force of the cardiac tissue was used as an approximation.

The force of contraction was measured in trabecula of freshly sacrificed adult chickens, using the same equipment and preparation methods as described by Lindgren and Altimiras [33]. Trabecula are bundles of muscle fibers running along the ventricular wall, and by carefully separating them from the rest of the muscle, minimal damage was inflicted upon the sample tissue. After dissection the trabecula were mounted with one end tied to a fixed rod and the other to a force displacement transducer, submerged in oxygenated, modified Ringer solution and stimulated with platinum electrodes to elicit contraction (frequency 1.5 Hz, pulse duration 20 ms and a voltage of 10-20 V). The trabecula were stabilized and adjusted to the muscle length that produced 80% of maximal twitch force, according to the procedures in [33]. This resting tension was maintained throughout

the experiment.

After preparation, the force of contraction of the trabecula was recorded for 5 minutes previous to the stimulation to attain a baseline value. Each trabeculae was then stimulated once for 10 min with 100 nM ISO to gain a maximal stimulation without overactivation (Isa Lindgren, unpublished values). The stimulation was interrupted by changing the buffer and the trabeculae was allowed to rest unperturbed for 5 minutes before being stimulated for 10 min with 100 nM ISO a second time.

The mean force of contraction was calculated for each minute of the recorded data. If the difference in force of contraction between 5 minutes and one minute before the stimulation was >30% the tissue was considered too unstable to give a reliable measurement. Likewise if the difference between one minute before the first stimulation and one minute before the second stimulation differed >50%.

The model was fitted to the data from this experiment and used to predict how the desensitisation behaved, and according to this prediction a second set of trials was carried out, with the resting time between the stimulations reduced to 2 minutes (see Figure 8).

4.7 Normalization Both experimental data and data simulated in the model were normalized with fold change where the last minute before the first stimulation was equal to 1. The variation was expressed as the standard error (SE).

4.8 Model requirements As mentioned previously, every model is a simplification of the real system and should be constructed to answer a certain question. Our model of the β-adrenergic signaling pathway is to be used when investigating the differences in the desensitisation of the system between fetal hearts and adults. As a step towards this, we first aim to construct a model that is capable of describing the adult desensitisation. To evaluate how good the model is a list of analytical tests and requirements have been formulated:

- Using data from the literature to perform a qualitative analysis to investigate if the model can perform the general desensitisation behavior of the β adrenergic pathway seen in previous studies [7]. This includes the peak effect and a prolonged resistance towards reactivation even though the stimulus have been removed briefly.

- It is assumed that the sensitivity of the cells to agonist stimulation will return after acute stimulation if the receptor is allowed to rest for a sufficient amount of time [1]. This must be the case in the model simulation as well.

- Can we fit the model to the experimental data and find acceptable parameter sets?
- Can we use the model to predict the duration of the desensitisation in cardiac tissue?
- Can we use the model to formulate core predictions about the system and identify key mechanisms for the desensitisation?

4.9 Project limitations

- The time limit for the project was 40 weeks.
- Experimental data for the states in our experimental setup was unavailable, we only had access to literature values.
- Restrictions in time and laboratory equipment prevented us from measuring cAMP directly in the tissue. Instead we performed a pilot study, where the amount of cAMP was approximated through the contractional force of the heart tissue.

5 Results

5.1 Structure of the original model In the original model, each receptor is considered to have four states. The first state is when it is in the cell membrane and receptive to activation. In the second state, the receptor is activated by the ligand and starts to signal through Gs. This happens as soon as the ligand is present in the model. The receptor then enters the third state where it is phosphorylated and can no longer signal through Gs. However, in the case of the B2-receptor, it starts signaling through Gi instead. In the cell, the receptor itself may be phosphorylated at several different sites by different proteins which will have different effect, but in this model the receptor simply is or is not phosphorylated. From the phosphorylated state the receptor can go back to the first state, waiting to be activated once again, or it can proceed to be internalized in the fourth state. In the cell the latter is a longer term state, as the receptor binds to β-arrestin and is brought into the cell in calveolae to be degraded or circulated out to the surface again. Internalized receptors in a cell may or may not be able to still signal, but in this model they are considered unable to do so. Downregulation is not considered as it would have no effect in the studied time period.

The two G-proteins, Gs and Gi, each have two states, inactive or active. They are considered as already bound to the receptor and ready to be activated as soon as there is ligand present. In this model the supply of GDP is inexhaustible and the reactivation of the G-protein is instantaneous, therefore binding of GTP to the G-protein is not modeled as a separate state variable. The G-proteins then affect the transformation between the inactive or active state of AC. The activated AC in turn stimulates production of cAMP, which is added to a basal production already present. The two groups of kinases, GRK and PKA, are activated by cAMP and phosphorylate only the active state of the receptor. In addition, PKA has a negative effect on the cAMP concentration.

The in signal, H1 and H2, is here treated as parameters rather than variables, and have different coefficients, which would make it possible to simulate activation with different ligands that might activate the two types of receptor differently. Neither H1, H2 nor their coefficients were optimized.

A flowchart of the different states present in the model can be seen in Figure 2, tab. 2 presents the model ODEs and tab. 1 presents the parameters and their biological equivalents.

Table 1: *List of state variables in the model and their biological equivalents.*

List of state variables	
State	Biological equivalent
B1	β_1-receptor in membrane, bound to G-protein and ready to receive a ligand
B1act	β_1-receptor in membrane, bound to ligand and signaling through $G_{\alpha s}$
B1p	Phosphorylated β_1-receptor in membrane, unable to signal
B1int	Internalized β_1-receptor, unable to signal
B2	β_2-receptor in membrane, bound to G-protein and ready to recieve a ligand
B2act	β_2-receptor in membrane, bound to ligand and signaling through $G_{\alpha s}$
B2p	Phosphorylated β_2-receptor in membrane, bound to ligand and signaling through $G_{\alpha i}$
B2int	Internalized β_2-receptor, unable to signal
Gs	$G_{\alpha s}$ bound to receptor
Gsact	Unbound $G_{\alpha s}$, able to activate AC
Gs	$G_{\alpha i}$ bound to receptor
Gsact	Unbound $G_{\alpha i}$, able to inhibit AC
AC	Adenylyl cyclace unable to catalyze production of cAMP
ACact	Adenylyl cyclace able to catalyze production of cAMP
cAMP	cyclic AMP
GRK	G-protein coupled receptor kinase unable to phosphorylate the receptor
GRKact	G-protein coupled receptor kinase able to phosphorylate the receptor
PKA	Protein Kinase A unable to phosphorylate the receptor
PKAact	Protein Kinase A able to phosphorylate the receptor and associated proteins able to catalyze breakdown of cAMP
H	Ligand

Table 2: *State variable equations for the original model.*

$$
\begin{aligned}
&d(B1)/dt = k1d * B1int + k1c * B1p - kbas1 * B1 - B1 * (kiso1 + kip1) * H1\\
&d(B1act)/dt = kbas1 * B1 + B1 * (kiso1 + kip1) * H1 - k1a * B1act *\\
&\qquad\qquad *(kGRK * GRKact + PKAact)\\
&d(B1p)/dt = k1a * B1act * (kGRK * GRKact + PKAact) - (k1b + k1c) * B1p\\
&d(B1int)/dt = k1b * B1p - k1d * B1int
\end{aligned}
$$

$$
\begin{aligned}
&d(B2)/dt = k2d * B2int + k2c * B2p - kbas2 * B2 - B2 * (kiso2 + kip2 + kter) * H2\\
&d(B2act)/dt = kbas2 * B2 + B2 * (kiso2 + kip2 + kter) * H2 - k2a * B2act *\\
&\qquad\qquad *(kGRK * GRKact + PKAact)\\
&d(B2p)/dt = k2a * B2act * (kGRK * GRKact + PKAact) - (k2b + k2c) * B2p\\
&d(B2int)/dt = k2b * B2p - k2d * B2int
\end{aligned}
$$

$$
\begin{aligned}
&d(Gs)/dt = k3b * Gsact - k3a * Gs * (B1act + B2act)\\
&d(Gsact)/dt = k3a * Gs * (B1act + B2act) - k3b * Gsact
\end{aligned}
$$

$$
\begin{aligned}
&d(Gi)/dt = k3d * Giact - k3c * B2p * Gi\\
&d(Giact)/dt = k3c * B2p * Gi - k3d * Giact
\end{aligned}
$$

$$
\begin{aligned}
&d(AC)/dt = k4b * ACact - k4a * Gsact/(kinh + Giact) * AC\\
&d(ACact)/dt = k4a * Gsact/(kinh + Giact) * AC - k4b * ACact
\end{aligned}
$$

$$
d(cAMP)/dt = cAMP0 + ACact * k5 - kPKA * PKAact * cAMP
$$

$$
\begin{aligned}
&d(GRK)/dt = -cAMP * k6a * GRK + k6b * GRKact\\
&d(GRKact)/dt = cAMP * k6a * GRK - k6b * GRKact
\end{aligned}
$$

$$
\begin{aligned}
&d(PKA)/dt = -cAMP * k6c * PKA + k6d * PKAact\\
&d(PKAact)/dt = cAMP * k6c * PKA - k6d * PKAact
\end{aligned}
$$

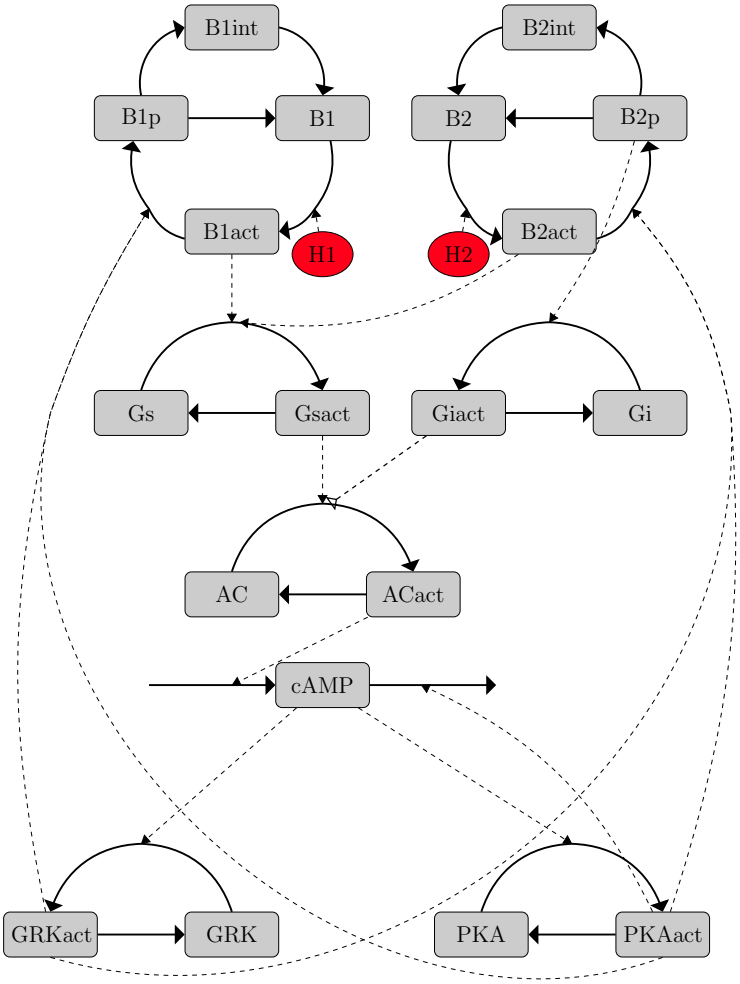


Figure 2: *Flow chart of the original model. Whole lines are transitions between different states of the same protein, dashed lines are catalytic actions with black arrow indication stimulation actions and white triangle inhibition.*

5.2 Minimal model structures Minimization of the model against the theoretical data showed that the desensitisation of the system could still be described if either the β_1 or the β_2 receptor was removed. The minimal model therefore has only one receptor, the β_1, which does not signal through $G_{\alpha i}$. Additionally, the internalized state of the receptor and the B1 state have been joined into one. The receptor thus have three states: an active state where it signals though $G_{\alpha s}$, a resting state where a ligand can bind and a phosphorylated state where it can neither be activated nor carry a signal. A model with a receptor of only two states is incapable of correctly describing the desensitisation.

Likewise, the Gs and the AC states can been joined without the system loosing the desensitisation.

Both phosphorylation of the receptor and breakdown of cAMP occurs spontaneously in the model, but the minimized model still requires a negative feedback loop from the kinases either to the receptor or to cAMP in order to describe the desensitisation behavior. Here the minimized model is split into two versions of a minimal model (Figure 3, tab. 3).

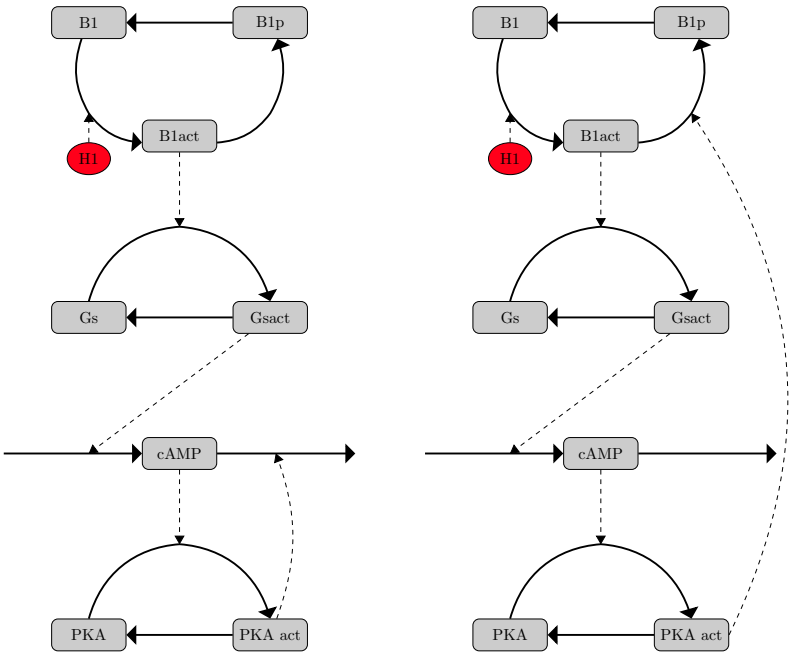
Table 3: *State variable equations for the minimal model.*

$$d(B1)/dt = k1c * B1p - kbas1 * B1 - B1 * (kiso1 + kip1) * H1$$
$$d(B1act)/dt = kbas1 * B1 + B1 * (kiso1 + kip1) * H1 - k1a * B1act$$
$$d(B1p)/dt = k1a * B1act - k1c * B1p$$

$$d(Gs)/dt = k3b * Gsact - k3a * Gs * B1act$$
$$d(Gsact)/dt = k3a * Gs * B1act - k3b * Gsact$$

$$d(cAMP)/dt = cAMP0 + Gsact * k5 - kPKA * PKAact * cAMP$$

$$d(PKA)/dt = -cAMP * k6c * PKA + k6d * PKAact$$
$$d(PKAact)/dt = cAMP * k6c * PKA - k6d * PKAact$$



(a) Minimal Model 1 (b) Minimal Model 2

Figure 3: *Minimal models of the system. One receptor with three receptor states and one feedback loop from the kinases either to the phosphorylation of the receptor or to breakdown of cAMP are both required and sufficient to describe the theoretical data.*

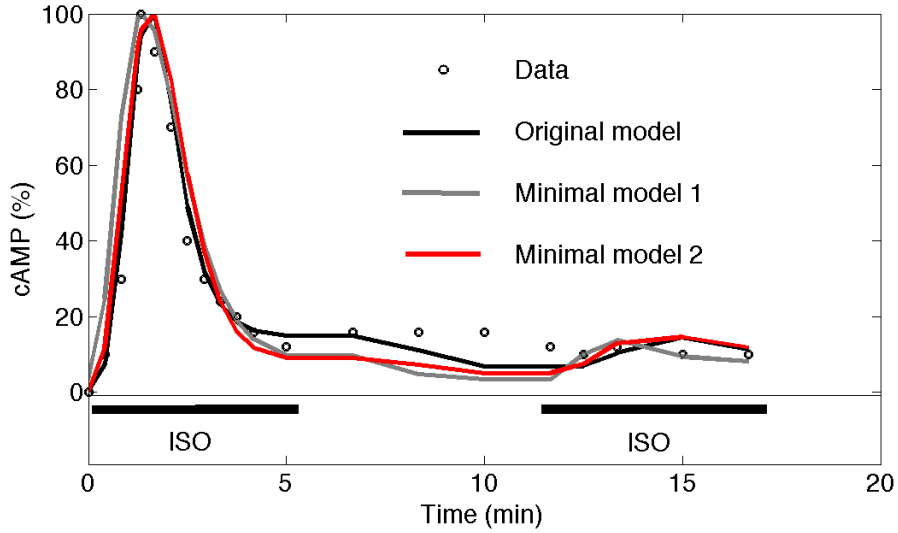


Figure 4: *Qualitative analysis: Models fitted to theoretical values adapted from experiments in [7] with a constant standard deviation.*

5.3 Qualitative fitting to theoretical desensitisation data In order to qualitatively evaluate the ability of a model to perform the desensitisation behavior it was fitted to data points adapted from an experiment by Xin *et al.* (Figure 4 [7]) with a constant standard error for each point. In this experiment Xin *et al.* estimated the cAMP content in HEK293 cells, an immortal cell line derived from human embryonic kidney cells, by measuring the current through transfected cyclic nucleotide-gated channels. The HEK-293 cells were stimulated with 1 μM ISO for 5 minutes, washed and allowed to rest during 5 minutes and then stimulated again for 5 minutes.

As can be seen in fig 4, the original model and the two minimal models are capable of performing the peak effect with its rapid rise and fall in response, as well as maintaining the desensitisation during a second stimulation. Simulations of the experiment where the resting time was changed also shows how the sensitivity to the second activation returns with a longer rest (Figure 5).

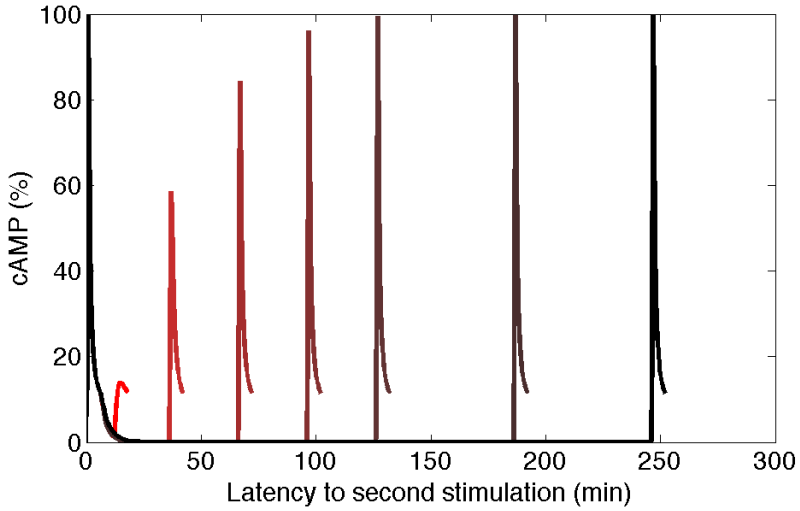


Figure 5: *All three models predict that the longer the cells are allowed to rest, the more forcefully they will respond to the second activation. Graph shows only the prediction from the original model.*

5.4 Cardiac contractility and model core prediction of a shorter resting time No significant difference in the amplitude of the peaks between the first and second stimulation could be discerned in our experimental setting, so clearly cardiac tissue resensitises with less than 5 minutes rest (Figure 6).

The models were optimized to the new data and all acceptable parameter sets found during the process was saved. The original model was able to correctly describe the experimental data, but the minimal models were not. Figure 7 shows the simulation of the experiment with the parameter set for the with the lowest cost found for the original model.

The acceptable parameter sets were then used to predict how short the rest between the two stimulations should be in order for us to catch the desensitisation. When allowed only one minute of rest, the simulation of the experiments showed no reaction to the second stimulation whereas a small peak could be seen for three minutes of rest (Figure 8).

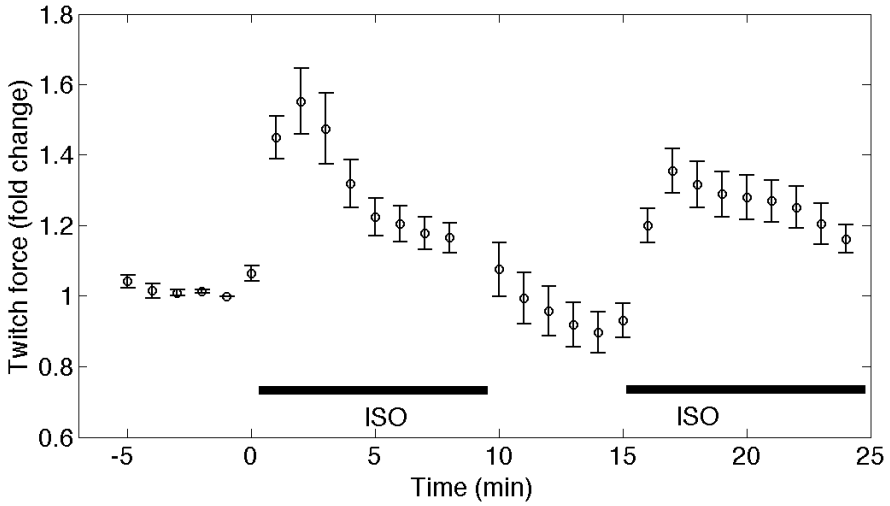


Figure 6: *Force of contraction in cardiac tissue. Normalized to fold change. o is mean of the trials and bars are SE (n=11).*

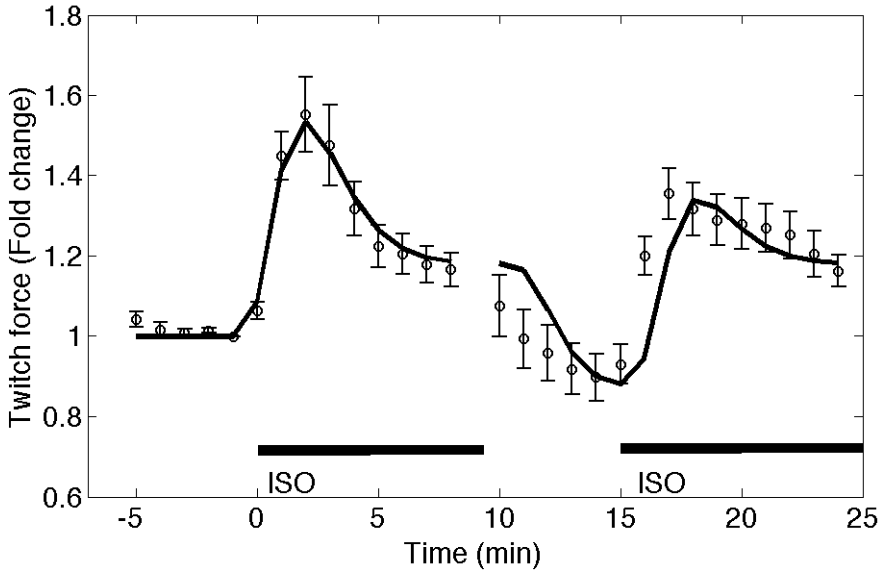
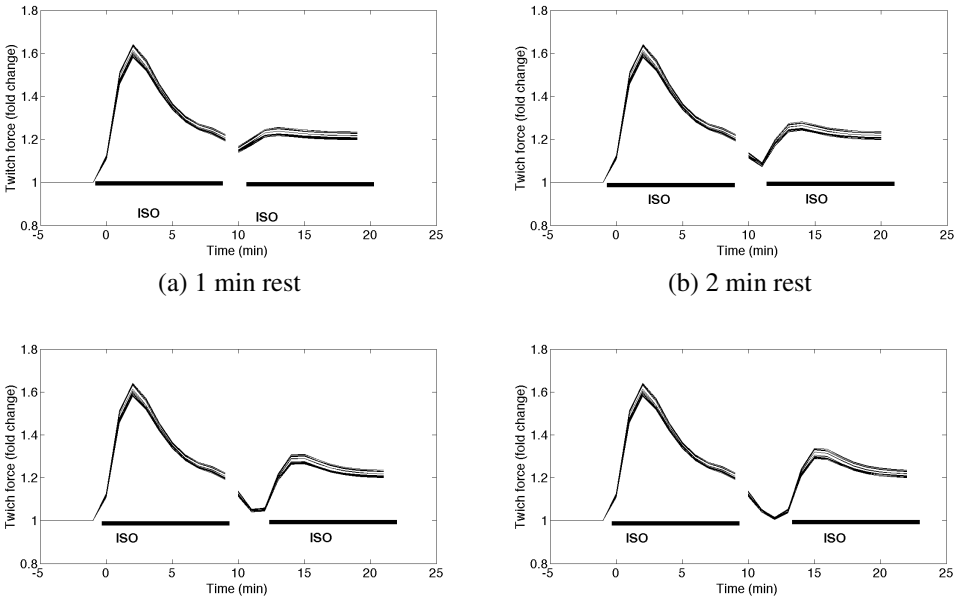


Figure 7: *Simulation of the experiment with the parameter set of the lowest cost.*



(a) 1 min rest (b) 2 min rest

(c) 3 min rest (d) 4 min rest

Figure 8: *Core predictions of 1-4 min rest (A-D respectively). The response to the second stimulation is completely gone when the rest is reduced to 1 min.*

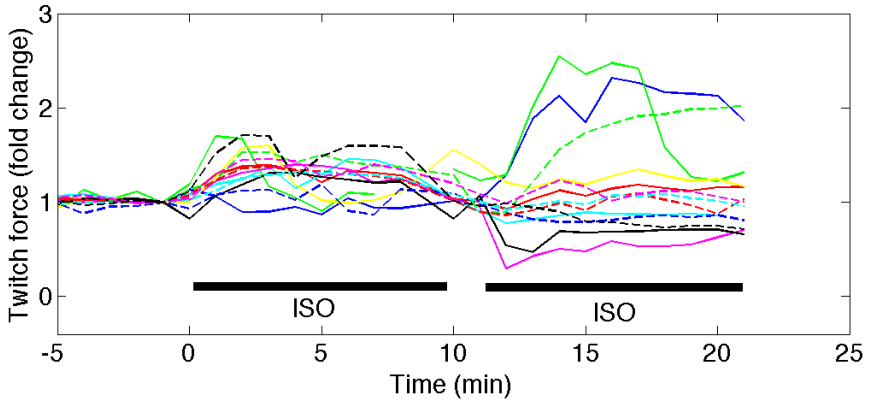


Figure 9: *Experimental testing of the model prediction with 2 min resting time (n=13). Each line represents one trial. The tissue was too disturbed during the washing and the prediction (Figure 8B) could neither be confirmed nor rejected.*

5.5 Testing of predicted resting time For all acceptable parameter sets found, the model predicted that the second peak would be almost non-existent, that is, the desensitisation still in effect, after a rest of only one minute (Figure 8A). However, the experimental setup did not allow for such a short resting time and instead we tested a two minute rest which the model predicted would also give a severely reduced second peak (Figure 8B).

With a resting time of only 2 minutes the behavior of the trabeculae at the second stimulation became too varied to give a conclusive image of the desensitisation (see Figure 9) and the prediction remains unconfirmed.

Table 4: *Summary of model capabilities.*

Model	Theoretical Data	Resensitisation	Experimental Data	Predict Resting Time
Original Model	Yes	Yes	Yes	Yes
Minimal Models	Yes	Yes	No	-
All Smaller Models	No	-	-	-

6 Discussion

The models in this thesis aim to describe the first steps in the signaling pathway of the cardiac β-adrenergic receptors. Already in the original version of the model several proteins and events have been lumped into the same state variables as a simplification (see 5.1), the most noteworthy of which are the phosphodiesterases activated by PKA, the β-arrestin binding to GRK as a part of the internalization process and several different isoforms of AC treated as the same protein. In this original version of the model, GRK and PKA phosphorylates the receptor through the same mechanism, even though in the cell they target different phosphorylation sites with a slightly different effect on the efficiency of for example the switching of the β_2 subtype from $G_{\alpha s}$ signaling to $G_{\alpha i}$ and the process of internalization.

Since we are only interested in the immediate desensitisation of the pathway, regulation of gene expression is not included in any of the models, and therefore up- and downregulation of the proteins participating in the pathway sets a time limit to the experiments that can be performed based on the predictions of the model. Neither is any type of crosstalk between receptors included. However, this

shows that according to our hypothesis of how the system works, no interference from sources outside of the system is needed to maintain the desensitisation.

Even more state variables have been joined or excluded in the minimal models. These models are only capable of describing the theoretical data, a minimal version that is also capable of describing the experimental data needs to be constructed. The exclusion of the β_2-receptor, and subsequently the $G_{\alpha i}$ pathway, shows that these two might not be critical to the desensitisation. This conclusion is supported by the fact that the β_2-receptor density is much lower than β_1-receptor density in the heart, and in rats the receptor type seems to be comparmentalized to the deep transverse tubules of the plasma membrane where they are hard to reach for the ligand [34]. The compartmentation of the signaling pathway changes during heart failure [35], and therefore this feature could be interesting to add in future versions of the model.

All three models are capable of qualitatively describing the de- and resensitisation of cardiac β-adrenoceptors, but only the original model could acceptably describe experimentally gained data where contraction of the tissue was used as an estimate of the cAMP production. The contraction of muscle tissue is a more complex process than synthesis of cAMP and is mostly influenced by calcium whose release is triggered not only via β-adrenoceptors [3]. Despite this, as a rough estimate of the cAMP production, the force of contraction is sufficient. The cardiac tissue displayed the typical peak behavior of the desensitisation during both the first and second stimulation and a stable baseline before the first stimulation. After washing, the baseline was lowered in most strips, and showed a greater variability. The mechanism behind this behavior is yet unidentified, but it could be caused by mechanical strain during washing. This could also be the cause of the great variation in response to the second stimulation after only two minutes of rest. Before the first stimulation the tissue was allowed to stabilize for at least 20 minutes, and two minutes might be too short for them to recover. In fact, several strips showed less stability in their resting tension after the washing in both experiments. The reason for the incapability of the minimal models to describe the experimental data seems to be connected to the fact that the force of contraction during the second stimulation starts at a lower baseline than the first one. The cost function considers each data point to be equally important, but we know that the tissue gets disturbed by the washing and that those data might not be as reliable as the peaks. The cost function could be weighted to reflect this. However, we get a good fit in the original model despite not performing these adjustments and no better fit could be attained in neither the original nor the minimal models by optimization only towards the first peak. The original model could then be used to

consistently predict how the desensitisation proceeds with differing resting times in the experiment, although these predictions remains to be tested (Figure 8).

Both the original model and the minimal models have been optimized only towards data of cAMP even though many other states have been included in the model structure. This makes the models over parameterized and unable to uniquely determine an acceptable parameter set. It is meant to provide an overview of the system that can help us identify where the most important mechanisms for the difference between fetal and adult signaling is located, and provide a basis for a more detailed investigation. If the remaining states were investigated, they might yield core predictions helping us identify the origin of the desensitisation, otherwise experimental data would need to be added to the model in order to make reliable predictions.

In the minimal models GRK phosphorylation of the receptor has been removed and despite this the model can still describe the desensitisation. Auman [1] claims that the first part of the cardiac β-adrenergic desensitisation is mediated through the β-adrenergic pathway itself but that the prolonged resistance to activation is due to interference from proteins activated by other receptors. However, Xin *et al* [7] show that in HEK293 cells, cAMP breakdown by PDE4, a cyclic nucleotide phosphodiesterase activated by PKA, is most responsible for the peak effect, while receptor phosphorylation by GRK is the main mechanism for retaining the desensitisation through the washing, and results from Violin *et al* supports this [28]. Xin *et al* [7] also show that the $G_{\alpha i}$ pathway does not significantly affect the cAMP signal. The result of the simulations is not surprising, however, as in this model GRK and PKA phosphorylates the receptor in the same way, which is not the case in the living cell. In improved versions of this model, GRK ought to be tied closer to the internalization of the receptor.

The minimal models also tells us that at least one feedback loop from the kinases, either to catalyze phosphorylation of the receptor or breakdown of cAMP, is both needed and sufficient in order to describe the desensitisation. To test this experimentally, phosphorylation of the receptor could be blocked, however it is the authors belief that adjustment of the GRK phosphorylation in the model should be carried out before any strong conclusions can be drawn from this result. Another interesting conclusion that can be drawn from the minimal models is that the remaining receptor needs three states. Merely switching the signaling through $G_{\alpha s}$ on or off is not enough, but some form of resting state needs to be included.

Future development and investigation of the model might include:

- Tie GRK phosphorylation of the receptor closer to internalization, as it has

been shown that the receptor is affected differently by phosphorylation by GRK and PKA, since they in real life phosphorylate at different sites.

- Fitting of the model to experimental data on the concentrations of cAMP in adult and fetal chicken hearts. This can be done in two ways: Fitting of the model to experimental data from fetal hearts with free parameters to investigate if the system structure can simulate the lack of desensitisation. Fitting of the model to experimental data from fetal hearts with the optimal parameter set from the adult model and only one or a few free parameters, to investigate if theoretical changes leading to the disappearance of the desensitisation can be identified.
- Inclusion of parameters that allow changes in the proportions between the proteins, not only between the different states of the same protein.
- Model reduction to find a minimal model for the experimental data.
- Inclusion of compartmentation in the model.

7 Conclusions

One feedback loop from the kinases to either the phosphorylation of the receptor or the breakdown of cAMP is necessary for the model to be able to describe the desensitisation, and three states in one single receptor is both enough and required.

The cardiac tissue displays the peak behavior of the desensitisation when stimulated with ISO for ten minutes. Five minutes of rest between stimulations is enough to allow it to resensitise. The cardiac tissue is predicted to be completely desensitised during the second stimulation if it is allowed to rest only one minute between stimulations.

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A The code

A.1 The models

Original model

```
********** MODEL NAME
beta1_2ver1

********** MODEL NOTES


********** MODEL STATES

d/dt(B1) = k1d*B1int + k1c*B1p - kbas1*B1 - B1*(kiso1 + kip1)*H1
d/dt(B1act) = kbas1*B1 + B1*(kiso1 + kip1)*H1 - k1a*B1act*(kGRK*GRKact+PKAact)
d/dt(B1p) = k1a*B1act*(kGRK*GRKact+PKAact) - (k1b+k1c)*B1p
d/dt(B1int) = k1b*B1p - k1d*B1int

d/dt(B2) = k2d*B2int + k2c*B2p - kbas2*B2 - B2*(kiso2 + kip2 + kter)*H2
d/dt(B2act) = kbas2*B2 + B2*(kiso2 + kip2 + kter)*H2 - k2a*B2act*(kGRK*GRKact+PKAact)
d/dt(B2p) = k2a*B2act*(kGRK*GRKact+PKAact) - (k2b+k2c)*B2p
d/dt(B2int) = k2b*B2p - k2d*B2int

d/dt(Gs) = k3b*Gsact - k3a*Gs*(B1act+B2act)
d/dt(Gsact) = k3a*Gs*(B1act+B2act) - k3b*Gsact
d/dt(Gi) = k3d*Giact - k3c*B2p*Gi
d/dt(Giact) = k3c*B2p*Gi - k3d*Giact

d/dt(AC) = k4b*ACact - k4a*Gsact/(kinh+Giact)*AC
d/dt(ACact) = k4a*Gsact/(kinh+Giact)*AC - k4b*ACact

d/dt(cAMP) = cAMP0 + ACact*k5 - kPKA*PKAact*cAMP

d/dt(GRK) = - cAMP*k6a*GRK + k6b*GRKact
d/dt(GRKact) = cAMP*k6a*GRK - k6b*GRKact
d/dt(PKA) = - cAMP*k6c*PKA + k6d*PKAact
d/dt(PKAact) = cAMP*k6c*PKA - k6d*PKAact
```

```
B1(0) = 7
B1act(0) = 1
B1p(0) = 1
B1int(0) = 1

B2(0) = 7
B2act(0) = 1
B2p(0) = 1
B2int(0) = 1

Gs(0) = 9
Gsact(0) = 1
Gi(0) = 9
Giact(0) = 1

AC(0) = 9
ACact(0) = 1
cAMP(0) = 1
GRK(0) = 9
GRKact(0) = 1
PKA(0) = 9
PKAact(0) = 1


********** MODEL PARAMETERS

kbas1 = 0.00001
kbas2 = 0.00001

k1a = 1
k1b = 1
k1c = 1
k1d = 1

k2a = 1
k2b = 1
k2c = 1
k2d = 1
```

```
k3a = 1
k3b = 1
k3c = 1
k3d = 1

k4b = 1
k4a = 1


k6a = 1
k6b = 1
k6c = 1
k6d = 1

kinh = 1
k5 = 1
kGRK = 1
kPKA = 1
cAMP0 = 1


kiso1 = 1
kiso2 = 1
kter = 1
kip1 = 1
kip2 = 1

H1 = 0
H2 = 0

********** MODEL VARIABLES

B1tot = B1int + B1act + B1 + B1p

B2tot = B2int + B2act + B2 + B2p

Bmem =  B1act + B1 + B1p + B2act + B2 + B2p
```

```
********** MODEL REACTIONS


********** MODEL FUNCTIONS


********** MODEL EVENTS


********** MODEL MATLAB FUNCTIONS
```

Minimal model 1

```
********** MODEL NAME
beta1_2verMini2

********** MODEL NOTES


********** MODEL STATES

d/dt(B1) = k1c*B1p - kbas1*B1 - B1*kiso1*H1
d/dt(B1act) = kbas1*B1 + B1*kiso1*H1 - k1a*B1act
d/dt(B1p) = k1a*B1act - k1c*B1p


d/dt(Gs) = k3b*Gsact - k3a*Gs*B1act
d/dt(Gsact) = k3a*Gs*B1act - k3b*Gsact


d/dt(cAMP) = cAMP0 + Gsact*k5 - kPKA*PKAact*cAMP


d/dt(PKA) = - cAMP*k6c*PKA + k6d*PKAact
d/dt(PKAact) = cAMP*k6c*PKA - k6d*PKAact
```

```
B1(0) = 8
B1act(0) = 1
B1p(0) = 1


Gs(0) = 9
Gsact(0) = 1

cAMP(0) = 1

PKA(0) = 9
PKAact(0) = 1


********** MODEL PARAMETERS

kbas1 = 0.00001
kbas2 = 0.00001

k1a = 1
k1b = 1
k1c = 1


k3a = 1
k3b = 1

k6c = 1
k6d = 1

k5 = 1
kPKA = 1
cAMP0 = 1


kiso1 = 1


H1 = 0
```

```
********** MODEL VARIABLES



********** MODEL REACTIONS

********** MODEL FUNCTIONS

********** MODEL EVENTS

********** MODEL MATLAB FUNCTIONS
```

Minimal model 2

```
********** MODEL NAME
beta1_2verMini3

********** MODEL NOTES


********** MODEL STATES

d/dt(B1) = k1c*B1p - kbas1*B1 - B1*kiso1*H1
d/dt(B1act) = kbas1*B1 + B1*kiso1*H1 - k1a*B1act*PKAact
d/dt(B1p) = k1a*B1act*PKAact - k1c*B1p


d/dt(Gs) = k3b*Gsact - k3a*Gs*B1act
d/dt(Gsact) = k3a*Gs*B1act - k3b*Gsact
```

```
d/dt(cAMP) = cAMP0 + Gsact*k5 - kPKA*cAMP


d/dt(PKA) = - cAMP*k6c*PKA + k6d*PKAact
d/dt(PKAact) = cAMP*k6c*PKA - k6d*PKAact



B1(0) = 8
B1act(0) = 1
B1p(0) = 1


Gs(0) = 9
Gsact(0) = 1

cAMP(0) = 1

PKA(0) = 9
PKAact(0) = 1


********** MODEL PARAMETERS

kbas1 = 0.00001
kbas2 = 0.00001

k1a = 1
k1b = 1
k1c = 1


k3a = 1
k3b = 1

k6c = 1
k6d = 1

k5 = 1
kPKA = 1
```

```
cAMP0 = 1

kiso1 = 1

H1 = 0

********** MODEL VARIABLES



********** MODEL REACTIONS

********** MODEL FUNCTIONS

********** MODEL EVENTS

********** MODEL MATLAB FUNCTIONS
```

A.2 The cost functions

The original model, theoretical data

```
function [error] = newCostFunctionAllStates(param,shouldIPlot)


    global icOrig
    global pNamesOpt
```

```
global modelName
global FID



modelName = 'beta1_2ver1';


simOptions = [];
simOptions.method = 'stiff';
simOptions.maxnumsteps = 1e6;
simOptions.abstol = 1e-9;
simOptions.reltol = 1e-9;

paramOrig = param;

H1 = 0;
H2 = 0;


paramSteady = [param H1 H2];

simDataSteadyState = SBPDsimulate(modelName,1000,icOrig,pNamesOpt,
paramSteady,simOptions);
newIC = simDataSteadyState.statevalues(end,:);
tmpError = 0;



\%\%\%\% Experiment from Xin, Clark, Tran, Rich 2008 \%\%\%\%
\%\% fig 4, control
\% plotted in fig 1

load('TestDataXin2008.mat')
measuredTimePoints = TestDataXin2008.Time;


H1 = 100;
H2 = 100;
param = paramOrig;
```

```
param(end-2:end) = 0;
paramXin2008 = [param  H1 H2];


try
    simDataXin2008_act1 = SBPDsimulate(modelName,measuredTimePoints(1:13),
    newIC,pNamesOpt,paramXin2008,simOptions);
catch exception
    disp(exception.message)
    disp('Now the simulation crashed in Xin, first stimulation');
    error = inf;
return
end



simDataXin2008_act1.statevalues(end,:);
peak = max(simDataXin2008_act1.statevalues(:,15));
SIMDATAXin2008 = simDataXin2008_act1.statevalues(:,15)./peak*100;


H1 = 0;
H2 = 0;
newSim = simDataXin2008_act1.statevalues(end,:)';

param = paramOrig;
param(end-2:end) = 0;
paramXin2008 = [param  H1 H2];


try
    simDataXin2008_wash = SBPDsimulate(modelName,measuredTimePoints(14:16),
    newSim,pNamesOpt,paramXin2008,simOptions);
catch exception
    disp(exception.message)
    disp('Now the simulation crashed in Xin, washout');
    error = inf;
return
end
```

```
rest = simDataXin2008_wash.statevalues(:,15)./peak*100;
SIMDATAXin2008 = [SIMDATAXin2008; rest];


H1 = 100;
H2 = 100;
newSim = simDataXin2008_wash.statevalues(end,:)';

param = paramOrig;
param(end-2:end) = 0;
paramXin2008 = [param  H1 H2];

try
    simDataXin2008_act2 = SBPDsimulate(modelName,measuredTimePoints(17:end),
    newSim,pNamesOpt,paramXin2008,simOptions);
catch exception
    disp(exception.message)
    disp('Now the simulation crashed in Xin, second stimulation');
    error = inf;
return
end


rest = simDataXin2008_act2.statevalues(:,15)/peak*100;
SIMDATAXin2008 = [SIMDATAXin2008; rest];



tmpError = tmpError + sqrt(sum((TestDataXin2008.Values-SIMDATAXin2008).^2./
(TestDataXin2008.Std.^2)));


AllStates = [simDataXin2008_act1.statevalues' simDataXin2008_wash.statevalues'
simDataXin2008_act2.statevalues'];


 if tmpError < 500
     fprintf(FID,'\%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f
     \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f
```

```
        \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f 4.10f \%4.10f \%4.10f \%4.10f
        \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \n',
        [tmpError, param]); fprintf(FID,'\n');
     end


  error = tmpError;


    if (nargin>1) & (shouldIPlot == 1)


    figure(1)
    plot(TestDataXin2008.Time,TestDataXin2008.Values,'ko')
    hold on
    plot(TestDataXin2008.Time,SIMDATAXin2008,'k-')
    xlabel('Time')
    ylabel('cAMP')


    end

end
```

The original model, experimental data

```
function [error] = CostFunctionAllStates137Data(param,shouldIPlot)



    global icOrig
    global pNamesOpt
    global modelName
    global FID
    global OptimData


    modelName = 'beta1_2ver1';
```

```
simOptions = [];
simOptions.method = 'stiff';
simOptions.maxnumsteps = 1e6;
simOptions.abstol = 1e-9;
simOptions.reltol = 1e-9;

paramOrig = param;



H1 = 0;
H2 = 0;

paramSteady = [param H1 H2];

simDataSteadyState = SBPDsimulate(modelName,1000,icOrig,pNamesOpt,
paramSteady,simOptions);
newIC = simDataSteadyState.statevalues(end,:);
tmpError = 0;



\%\%\%\% Egna experiment \%\%\%\%
\%\% 10 min stimulation, 5 min rest, 10 min stimulation
\% plotted in fig 1

measuredTimePoints = (0:10/900:10)';
measurements = OptimData(2:30,13:15);



H1 = 0;
H2 = 0;
param = paramOrig;
param(end-2:end) = 0;
paramData5min_rest1 = [param  H1 H2];
```

```
try
    simData5min_rest1 = SBPDsimulate(modelName,measuredTimePoints(1:450),
    newIC,pNamesOpt,paramData5min_rest1,simOptions);
catch exception
    disp(exception.message)
    disp('Now the simulation crashed during the first rest');
    error = inf;
return
end

SimMeasure1 = [mean(simData5min_rest1.statevalues(1:90,15))
mean(simData5min_rest1.statevalues(91:180,15))
mean(simData5min_rest1.statevalues(181:270,15))
mean(simData5min_rest1.statevalues(271:360,15))
mean(simData5min_rest1.statevalues(361:450,15))];


norm = SimMeasure1(end);
 SIMDATA = SimMeasure1./norm;



H1 = 100;
H2 = 100;
newSim = simData5min_rest1.statevalues(end,:)';
param = paramOrig;
param(end-2:end) = 0;
paramData5min_act1 = [param  H1 H2];


try
    simData5min_act1 = SBPDsimulate(modelName,measuredTimePoints(1:900),
    newSim,pNamesOpt,paramData5min_act1,simOptions);
catch exception
    disp(exception.message)
    disp('Now the simulation crashed during the first stimulation');
    error = inf;
return
end
```

```
SimMeasure2 = [mean(simData5min_act1.statevalues(1:90,15))
mean(simData5min_act1.statevalues(91:180,15))
mean(simData5min_act1.statevalues(181:270,15))
mean(simData5min_act1.statevalues(271:360,15))
mean(simData5min_act1.statevalues(361:450,15))
mean(simData5min_act1.statevalues(451:540,15))
mean(simData5min_act1.statevalues(541:630,15))
mean(simData5min_act1.statevalues(631:720,15))
mean(simData5min_act1.statevalues(721:810,15))
mean(simData5min_act1.statevalues(811:900,15))];

act1 = SimMeasure2./norm;

 SIMDATA = [SIMDATA act1];



 H1 = 0;
 H2 = 0;
 newSim = simData5min_act1.statevalues(end,:)';

 param = paramOrig;
 param(end-2:end) = 0;
 paramData5min_rest2 = [param  H1 H2];


 try
     simData5min_rest2 = SBPDsimulate(modelName,measuredTimePoints(1:450),
     newSim,pNamesOpt,paramData5min_rest2,simOptions);
 catch exception
     disp(exception.message)
     disp('Now the simulation crashed during the second rest');
     error = inf;
 return
 end

 SimMeasure3 = [mean(simData5min_rest2.statevalues(1:90,15))
```

```
mean(simData5min_rest2.statevalues(91:180,15))
mean(simData5min_rest2.statevalues(181:270,15))
mean(simData5min_rest2.statevalues(271:360,15))
mean(simData5min_rest2.statevalues(361:450,15))];


rest2 = SimMeasure3./norm;
SIMDATA = [SIMDATA rest2];


H1 = 100;
H2 = 100;
newSim = simData5min_rest2.statevalues(end,:)';

param = paramOrig;
param(end-2:end) = 0;
paramData5min_act2 = [param  H1 H2];

try
    simData5min_act2 = SBPDsimulate(modelName,measuredTimePoints(1:900),
    newSim,pNamesOpt,paramData5min_act2,simOptions);
catch exception
    disp(exception.message)
    disp('Now the simulation crashed during the second stimulation');
    error = inf;
return
end

SimMeasure4 = [mean(simData5min_act2.statevalues(1:90,15))
mean(simData5min_act2.statevalues(91:180,15))
mean(simData5min_act2.statevalues(181:270,15))
mean(simData5min_act2.statevalues(271:360,15))
mean(simData5min_act2.statevalues(361:450,15))
mean(simData5min_act2.statevalues(451:540,15))
mean(simData5min_act2.statevalues(541:630,15))
mean(simData5min_act2.statevalues(631:720,15))
mean(simData5min_act2.statevalues(721:810,15))
mean(simData5min_act2.statevalues(811:900,15))];
```

```
    act2 = SimMeasure4./norm;
    SIMDATA = [SIMDATA act2];
    tmpError = tmpError + sum((measurements([1:4 6:29],1)-
    SIMDATA([1:4 6:14 16:30])').^2./(measurements([1:4 6:29],3).^2));

    AllStates = [simData5min_rest1.statevalues' simData5min_act1.statevalues'
    simData5min_rest2.statevalues' simData5min_act2.statevalues'];



    if tmpError < chi2inv(0.95,28)
           fprintf(FID,'\%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f
           \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f
           \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f
           \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \n',
           [tmpError, param]); fprintf(FID,'\n');
    end


  error = tmpError;


    if (nargin>1) & (shouldIPlot == 1)

SimulatedData = SIMDATA'


    figure(1)
    errorbar(OptimData(2:30,1),measurements(:,1),measurements(:,3),'b*')
    hold on
    plot(OptimData(2:15,1),SIMDATA(1:14),'b-')
    hold on
    plot(OptimData(16:30,1),SIMDATA(15:29),'b-')
    xlabel('Time')
    ylabel('Twitch force (Fold change)')


    end

end
```

The minimal models, theoretical data

```
function [error] = newCostFunctionAllStatesMini(param,shouldIPlot)



    global icOrig
    global pNamesOpt
    global modelName
    global FID


     modelName = 'beta1_2verMini2';
    \% modelName = 'beta1_2verMini3';

    simOptions = [];
    simOptions.method = 'stiff';
    simOptions.maxnumsteps = 1e6;
    simOptions.abstol = 1e-9;
    simOptions.reltol = 1e-9;

    paramOrig = param;

    H1 = 0;

    paramSteady = [param H1];

    simDataSteadyState = SBPDsimulate(modelName,1000,icOrig,pNamesOpt,
    paramSteady,simOptions);
    newIC = simDataSteadyState.statevalues(end,:);
    tmpError = 0;



    \%\%\%\%\%Experiment from Xin, Clark, Tran, Rich 2008 \%\%\%\%\%
    \%\% fig 4, control
```

```
\% plotted in fig 1

load('TestDataXin2008.mat')
measuredTimePoints = TestDataXin2008.Time;


H1 = 100;
param = paramOrig;
paramXin2008 = [param  H1];


try
    simDataXin2008_act1 = SBPDsimulate(modelName,measuredTimePoints(1:13),
    newIC,pNamesOpt,paramXin2008,simOptions);
catch exception
    disp(exception.message)
    disp('Now the simulation crashed in Xin, first stimulation');
    error = inf;
return
end


peak = max(simDataXin2008_act1.statevalues(:,6));
SIMDATAXin2008 = simDataXin2008_act1.statevalues(:,6)./peak*100;


H1 = 0;
newSim = simDataXin2008_act1.statevalues(end,:)';

param = paramOrig;
paramXin2008 = [param  H1];


try
    simDataXin2008_wash = SBPDsimulate(modelName,measuredTimePoints(14:16),
    newSim,pNamesOpt,paramXin2008,simOptions);
catch exception
    disp(exception.message)
    disp('Now the simulation crashed in Xin, washout');
    error = inf;
```

```
return
end


rest = simDataXin2008_wash.statevalues(:,6)./peak*100;
SIMDATAXin2008 = [SIMDATAXin2008; rest];


H1 = 100;
newSim = simDataXin2008_wash.statevalues(end,:)';

param = paramOrig;
paramXin2008 = [param  H1];

try
    simDataXin2008_act2 = SBPDsimulate(modelName,measuredTimePoints(17:end),
    newSim,pNamesOpt,paramXin2008,simOptions);
catch exception
    disp(exception.message)
    disp('Now the simulation crashed in Xin, second stimulation');
    error = inf;
return
end


rest = simDataXin2008_act2.statevalues(:,6)/peak*100;
SIMDATAXin2008 = [SIMDATAXin2008; rest];



tmpError = tmpError + sqrt(sum((TestDataXin2008.Values-SIMDATAXin2008).^2./
(TestDataXin2008.Std.^2)));


AllStates = [simDataXin2008_act1.statevalues' simDataXin2008_wash.statevalues'
simDataXin2008_act2.statevalues'];


  if tmpError < 500
      fprintf(FID,'\%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f
```

```
            \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \%4.10f \n',
            [tmpError, param]); fprintf(FID,'\n');
        end


    error = tmpError;


      if (nargin>1) & (shouldIPlot == 1)


      figure(1)
      plot(TestDataXin2008.Time,TestDataXin2008.Values,'ko')
      hold on
      plot(TestDataXin2008.Time,SIMDATAXin2008,'r-')
      xlabel('Time')
      ylabel('cAMP')


      end

end
```